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Subacute toxicity of uranyl acetate in Swiss-Albino mice

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Abstract

Subacute effects of uranyl acetate were investigated in laboratory mice (*Mus musculus*, Swiss-Albino). Uranyl acetate was administrated to mice during a period of 5 days with dietary consumption ad libitum. Effects of uranyl acetate on food and water consumption, body weight changes; plasma urea nitrogen (BUN), creatinine concentrations and activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by time-course experiment. Glutathione *S*-transferase (GST) and catalase (CAT) activities were also determined in liver tissues on day 5. Distribution of radioactivity in liver, kidney and brain was detected by scintillation spectrometry. The results indicated that uranyl acetate was accumulated in examined tissues, with highest accumulation being in brain. Some of the biochemical biomarkers (BUN, creatinine, ALP) were significantly increased ($P < 0.05$) in the exposure group compared to control animals. Also, BUN and/or creatinine levels and/or ALT and AST activities significantly increased ($P < 0.01$ or $P < 0.05$) with UA exposure on day 3 and/or day 5 compared with results of day 1.

Keywords: Uranyl acetate; Toxicity; Biomarker; Mice

1. Introduction

Uranium is a naturally occurring element in earth. The best known area of use of this element is in the nuclear weapons field and its use as a fuel in nuclear power reactors. The by-products formed when processing uranium into nuclear fuel are especially dangerous to those people directly involved in the industrial processes (De Rey et al., 1983; Llobet et al., 1991). The increasing role of this metal in nuclear industry results in an increasing occupational exposure to uranium and puts the general population at risk for chronic exposure to low levels of uranium ions by inhalation or by dietary intake (Bosque et al., 1993a,b). The kidney is a common target organ for toxicity of uranium and of numerous other substances due to the magnitude of blood flow through it (McDonald-Taylor et al., 1992). Morphological alterations in the kidney associated with uranium and/or their uranyl ion toxicity primarily involve the renal corpuscle and proximal tubule (Avasthi et al., 1980). In nature, uranium is commonly found as uranyl ion (UO_2) which is the form in which it is present in the mammalian body (La Touche et al., 1987). Intravenous or oral administration of uranyl ions is the easiest and most efficient procedure to create acute renal failure (ARF) by causing necrosis of the distal portion of the proximal renal tubules (Mahmood and Waters, 1994). It was reported that maximal effect is seen 5 days after the uranyl nitrate administration to rats (Giacomini et al., 1981). The uranium salts did not affect only renal failure, but also enzymeuria, glycosuria, aminoaciduria and proteinuria were reported to occur after acute uranium poisoning (Blantz, 1975; Bentley et al., 1985; Domingo et al., 1987, 1989; Anthony et al., 1994). Acute oral LD_{50} was detected as 250 mg/kg for mice in previous toxicity studies for uranyl acetate (UA) (Domingo et al., 1987).

The aim of this study is the evaluation of some biochemical biomarkers due to subacute toxic effect of

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uranyl salt and also accumulation levels of the radioactive ions from the selected tissues of albino mice. Uranium was administered as UA in the present investigation.

2. Materials and methods

In the present study, 18 male mice (*Mus musculus*, Swiss-Albino) weighing initially from 20 to 30 g were used. The mice were kept in individual cages and maintained at 25 ± 2°C, and at 40–60% relative humidity. Mice fed ad libitum with a 12:12-h light:dark cycle. Grounded mice chow (Elazıg Yem Sanayi, Turkey) was accessed through 5-cm diameter ports cut in the lids of 75-ml plastic bottles; tap water was provided ad libitum. Sand was used as bedding material to facilitate collection of spilled and uneaten food. The sand was sifted out and the remaining food weighed for calculation of food consumption in both acclimatization and treatment periods. Food and water consumption were recorded during the study for calculation of the amount of UA taken with food eaten by the animals (Ozmen et al., 1998). Animals were controlled and remained in acclimatization period for 8 days before treatment with uranyl acetate [$(CH_3COO)_2 \cdot UO_2 \cdot 2H_2O$].

Two grams of UA were dissolved in 12 ml distilled water and 16 ml corn oil was added to the mixture. All chemicals were analytical grade and purchased from Merck Chemical Corporation (Germany). The solution was added to 400 g of grounded commercial mice chow; the final concentration of UA was 0.5% in the diet. Mice were divided, randomly, into two equal groups. Group 1 was the control and they were fed a diet containing corn oil and distilled water only, without uranyl acetate. The other group (Group 2) was fed with UA-containing food. Mice of both groups were starved for 12 h before being exposed to dietary UA.

Blood samples were collected during day 1, and on days 3 and 5 of treatment with UA, from the retro-orbital sinus of mice according to the method of Ozmen et al. (1995). Blood was centrifuged at 2400 × *g* for 10 min at 4°C. After centrifugation, plasma was stored at −40°C for biochemical assays.

Mice were sacrificed by cervical dislocation on day 5 of the treatment, and liver, brain and kidney were removed quickly. Tissues were frozen in liquid nitrogen and kept at −40°C. Liver was perfused with potassium phosphate buffer (0.1 M, pH 7.2) and homogenized in ice-cold phosphate buffer (0.1 M, pH 7.2; containing 0.05% Triton X-100) using a polythrone homogenizer (Status, UK) and then sonicated for 15 s in a crushed-ice container using a sonifier (Bronson 450, USA). The homogenate was centrifuged at 16000 × *g* for 15 min at 4°C. The supernatant was used for glutathione *S*-transferase (GST) and catalase (CAT) activities (Lück, 1963; Habig et al., 1974). Total protein assay was conducted according to the method of Lowry et al. (1951). Plasma was used for analysis of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities, and for blood urea nitrogen (BUN) and creatinine concentrations. A UV/Vis spectrophotometer (Philips Inc., PU8790) was used for assay of GST, CAT and total protein from liver supernatant. Plasma samples were evaluated in the Beckman autoanalyser for ALP, ALT, AST, BUN and creatinine assays using Sigma Diagnostics (St. Louis, MO). Scintillation was determined by LS 5000 TD scintillation spectrometry (Beckman, USA). For this, a radioactivity determination was performed on day 5. Collected tissues were weighed and 100 μg of tissue sample was sonicated in phosphate buffer in a crushed-ice container. Homogenate was transferred to counting vials and 5 ml aquasol (Sigma, MO, USA) was added. Counting of radioactivity was performed for 10 min for each vial and results were calculated as cpm/g tissues.

Statistical analysis of data was carried out using SPSS for Windows statistical software (SPSS Inc., USA). Independent samples were analyzed by Student's *t*-test. Comparison of differences between test groups was done by Mann–Whitney *U*-test. Statistical significance was set at the 95% confidence level.

3. Results

The mean daily UA exposure has been calculated as 905 ± 40.0 mg/kg of body weight of mice with respect to daily mean food consumption. According to this, Table 1 shows radioactivity counts of the selected tissues of mice. The accumulation of the radioactivity in brain, kidney and liver has been found to be significantly different ($P < 0.05$) compared to the tissues of the control animals.

Table 2 shows mean food consumption and body weight changes of the pre- and post-treatment periods, and the daily mean water consumption for the control and exposure groups of animals. In these calculations,

Table 1
Radioactivity values of selected tissues of mice exposed to uranyl acetate

Experimental group	*n*	Radioactivity (cpm/g tissue)		
		Liver	Kidney	Brain
Control	9	989 ± 43.6	1246 ± 31.4	1054 ± 87.3
UA	9	1248 ± 100*	1344 ± 90.9*	1824 ± 454*

Values shown are means ± S.E.M. *n*, number of animals.
*Statistical differences, found between control and UA exposed animals ($P < 0.05$).

Table 2
Daily mean food and water consumption and degree of UA exposure of pre- and post-treatment mice

	Experimental group	
	Control ($n=9$)	UA ($n=9$)
UA exposure (mg/kg/day)	0	905 ± 40.0
Pre-treatment food consumption (g/kg/day)	180.6 ± 6.77	172.4 ± 6.58
Post-treatment food consumption (g/kg/day)	220.7 ± 9.28	162.9 ± 5.83*
Water consumption (ml/day/mouse)	5.32 ± 1.49	6.05 ± 1.96
Pre-treatment weight changes (g, %)	5.80 ± 1.40	5.18 ± 1.41
Total weight changes (g, %)	5.34 ± 0.84	−3.65 ± 1.73*

Values shown are means ± S.E.M. n, number of animals.
*Statistical differences, found between control and UA-exposed animals ($P<0.01$).

we found that there are no significant differences ($P>0.05$) between the experimental groups for food consumption during the pre-treatment period. However, UA exposure significantly ($P<0.01$) decreased the food consumption of the UA exposure group compared to control animals in the post-treatment period. The total mean body weight increased by 5.80 and 5.18% for control and treated animals, respectively, in the acclimatization period. However, UA exposure decreased mean total body weight by 3.65% in mice within 5 days while controls gained weight (5.34%) (Table 2). These results were also found to be significantly different ($P<0.01$) compared to the control animals. On the other hand, mortality did not occur during the experimental period in any of the test groups.

Biochemical biomarkers were also investigated to test the dietary subacute toxicity of UA in mice. For this reason, AST, ALT, ALP activities, and BUN and creatinine concentrations were studied from the plasma samples on day 1, and on days 3 and 5 of exposure. In addition to these biomarkers, GST and CAT activities of liver tissue were assayed from animals sacrificed on day 5. When the results were compared within control and treatment animals, UA significantly ($P<0.05$) increased BUN and creatinine concentrations in the exposed mice on day 1, day 3 and/or on day 5 (Table 3). Also, some biochemical markers showed significant differences within the UA exposure period (Table 3). ALT and AST activities were not changed with exposure period, but ALP was significantly ($P<0.05$) increased on days 3 and 5. On the other hand, CAT and GST specific activities of liver tissues were not changed in subacute UA-exposed mice.

4. Discussion

The studies of toxic subacute effects of uranium on mammals are limited in previous studies. Especially, chronic effects of uranium salts on the developmental stage of animals have been investigated. Also, it has been shown that uranium salts caused renal malfunction in these studies (Blantz, 1975; Avasthi et al., 1980; McDonald-Taylor et al., 1992, Schramm et al., 1994). BUN and creatinine levels have been increased during a time-course experiment in uranium salt-exposed animals (McDonald-Taylor et al., 1992; Anthony et al., 1994).

Kidney is the most important tissue to eliminate heavy metals. Significant impairments of renal functions are known to result from heavy metal exposure (such as with UA). This elimination of UA via kidneys diminished in time (Belpaire et al., 1993). In the present study, the subacute effects of UA have been investigated on mice using some biomarkers. Therefore, after giving one dose of uranyl acetate to animals the results were worked out according to a normalizing of data. Death was not observed in the UA-treated animals during the experiment. Our data were confirmed by the previous studies (Anthony et al., 1994; Mahmood and Waters, 1994).

Table 3
Summary of results of biochemical markers from the plasma samples and liver tissues of experimental groups on test days

Biochemical markers	Experimental group	
	Control	UA
Day 1		
BUN (mg/dl)	15.42 ± 0.42	17.33 ± 0.83*
Creatinine (mg/dl)	0.342 ± 0.043	0.667 ± 0.13[b]
ALT (U/ml)	17.62 ± 1.71	23.33 ± 1.22
AST (U/ml)	55.55 ± 3.42	50.66 ± 2.40
ALP (U/ml)	114.2 ± 10.8	107.6 ± 6.34
Day 3		
BUN (mg/dl)	18.75 ± 0.75	22.87 ± 1.95[b,**]
Creatinine (mg/dl)	0.814 ± 0.17	1.30 ± 0.10*
ALT (U/ml)	10.0 ± 1.06	14.28 ± 0.80**
AST (U/ml)	40.0 ± 2.00	50.28 ± 3.58
ALP (U/ml)	84.0 ± 3.90	113.3 ± 11.6*
Day 5		
BUN (mg/dl)	15.66 ± 0.61	28.40 ± 5.03[a,**]
Creatinine (mg/dl)	0.667 ± 0.04	0.733 ± 0.067
ALT (U/ml)	26.57 ± 1.61	30.0 ± 2.10*
AST (U/ml)	78.33 ± 6.31	92.75 ± 5.47*
ALP (U/ml)	86.60 ± 4.83	117.42 ± 10.9*
CAT (μmol/mg total protein)	0.0226 ± 0.003	0.0221 ± 0.001
GST (μmol/mg total protein)	3.226 ± 0.077	3.155 ± 0.060

Values shown are means ± S.E.M.
Statistical differences found between control and UA-exposed animals: [a] $P<0.05$; [b] $P<0.01$.
Indicates differences significant at various level according to exposure period only within UA-exposure group ($P<0.01$; **$P<0.05$).

The highest radioactivity value was found at brain samples of exposed mice. The radioactivity increased by 73.0% in brain tissue. But no behavioral changes were observed in mice with regard to neural functions. However, it was found that UA accumulation was maximum in the brain, while the kidney was the most damaged tissue by the chemical, as described in previous studies (McDonald-Taylor et al., 1992; Anthony et al., 1994).

Plasma BUN concentration was increased with UA administration. Also creatinine levels were increased in the duration of this experiment, and the differences were found to be significant ($P < 0.01$) between control and exposed animals on day 1. AST activity is known to be an indicator of muscle and heart diseases. The determination of ALT and ALP activities is the criterion in hepatic tissue necrosis. Also, ALP is an important enzyme in bone activities (Hoffmann et al., 1989).

Liver ALT and AST enzyme activities were not significantly ($P > 0.05$) changed between control and UA-treated animals during the experiment, but plasma ALP activity significantly ($P < 0.05$) increased during exposure to UA on days 3 and 5 compared to control animals (Table 3). There was no significant difference ($P > 0.05$) in the liver GST and CAT activities between control and treated animals. This situation may explain why subacute UA treatment did not cause accumulation of the chemical in liver tissues. Thus, the phase II detoxification mechanism was not affected by UA. CAT acts as a scavenger of toxic oxygen products in the aerobic metabolism (Fıskın and Asma-Hamamcı, 1996). On the other hand, GST plays an important role in removal of toxic substrates and in the phase II detoxification mechanism (Glenn and Gandolfi, 1991). In the present study, observation of body weight and depletion of food are also important indicators of UA toxicity.

Radioactivity in exposed animals significantly ($P < 0.05$) increased in liver, kidney and brain when compared with control mice. This can be attributed to the quantity of UA and the exposure period. The accumulation of UA in some tissues is an important criterion for evaluation of effects of UA. Uranium also causes environmental pollution, and organisms can be exposed to this pollutant because of its use as a fuel in power plants. Also, contamination of foods by uranium salts is another risk factor for living organisms.

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